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PATENT- OG VAREMÆRKESTYRELSEN

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TARGETING SINGLE EPITOPES

FIELD OF THE INVENTION

The present invention relates to the field of active specific immunotherapy ("therapeutic vaccination"). In particular, the present invention relates to novel immunogenic agents that are capable of inducing specific B-cell immunity that is directed against one single epitope present in a self-antigen. The invention further relates to methods of preparing such immunogens, methods of using the immunogens in therapy. Further, the invention relates to proteins, nucleic acid fragments, recombinantly modified host cells and virus that are useful in the practice of the methods of the invention.

BACKGROUND OF THE INVENTION

Active specific immunotherapy (also known as therapeutic vaccination) that targets weak antigens and self-proteins has attained increased interest in the last two decades. The idea of stimulating the immune system to target antigens relevant for a pathological state includes many attractive aspects in terms of patient compliance, safety and efficacy.

Traditionally, therapeutic vaccines have been prepared as chemical conjugates between an antigen and a large foreign carrier protein, where the latter provides for T-cell epitopes that are recognised by the immune system as being foreign. A refinement of this approach utilises fusion molecules where the fusion partners are constituted by the antigen (normally proteinaceous) and the foreign carrier. These two approaches share the drawback of utilising a large carrier molecule that

is more immunogenic than the self-protein and also includes a large number of B-cell epitopes, and hence the immune response typically matures into a response directed primarily against the carrier protein and consequently dosing of the vaccine has to be increased over time (a phenomenon known in the art as "carrier suppression" of the immune response). Further, the use of large fusion constructs has a tendency to mask epitopes in the antigen, thereby rendering the immune response even less relevant.

A related approach has been to couple a single defined supposed B-cell epitope (in the form of a short peptide) to either a single, broadly recognized, T helper epitope or to a carrier protein (the "hapten-carrier" approach). These approaches both suffer the drawback that the immune response against the single B-cell epitope is very hard to control, since the native conformation of a peptide stretch from a larger protein is very difficult to maintain. In general, such peptide vaccines have met a lot of problems due to the weak immune responses induced.

Other approaches include coupling of self-antigens or epitopes to non-immunogenic carriers, optionally in combination with foreign T_H epitope containing sequences, cf. e.g. WO 02/066056 which discloses non-immunogenic polyhydroxypolymer carriers whereto are bound B-cell epitopes and T_H epitopes. This approach, like other peptide based approaches, works fine if the peptide bound to the non-immunogenic carrier is a significant linear B-cell epitope, but for more complicated 3-dimensionally constrained epitopes, this approach suffers the drawback of not providing a means to constrain the epitope in an immunogenically relevant 3D conformation.

Another approach has been to prepare anti-idiotypic antibodies raised against antibodies that recognize an antigen it is of interest to down-regulate. To a large extent, this approach resembles the hapten-carrier approach where the non-idiotypic regions of the anti-idiotypic antibody serves as a carrier moiety, and where the idiotype of the anti-idiotypic antibody is a 3-dimensional mimic of an epitope of the antigen of interest. Again, the problem arises that the anti-idiotypic antibody is either foreign to the vaccinated animal, so the immune response will over time be directed towards the non-essential parts of the anti-idiotypic antibody, or the antibody is self and therefore the immune response will be very weak.

In WO 99/25378 it has been suggested to graft one member of a binding pair into the CDR (complementarity determining region) of an antibody and use this engineered antibody as an immunization agent - for all practical purposes, such an engineered antibody will share the same features as does a anti-idiotypic antibody. WO 99/25378 mentions no specifics that relate to the problems associated with using autologous vs. heterologous antibodies as recipients for the grafted molecule, *i.e.* there is no discussion of the problems of carrier suppression when using a heterologous antibody for vaccination or lack of immunogenicity when using an autologous antibody.

One way to overcome the above problems with carrier suppression was suggested in WO 95/05849 where use of minimally destructive immunogenizing modifications of self-proteins where brought about by introduction of single, defined T helper epitopes in the self-protein amino acid sequence while preserving tertiary structure of the self-protein. This approach provides for induction of immune

responses that target a majority of B-cell epitopes in the self-protein without the immune response being shifted towards a carrier moiety.

However, certain antigens are for various reasons difficult to target by means of vaccination that produces antibodies reactive with the complete molecule. For instance, the antigen may be a membrane protein where only minor parts are exposed to the extracellular phase and thereby accessible to antibody binding. Also, if it is of great concern to avoid activation of secondary immune mechanisms (complement, NK activity etc.) it is not suitable to direct a polyclonal antibody response against the antigen. Finally, certain antigens must for safety reasons be targeted in specific epitopic regions to avoid safety issues - for instance, IgE can be safely targeted in the FcεR binding region, whereas it is controversial whether other parts of the molecule can be targeted in a clinical setting.

Such antigens can be targeted with specific passive immunotherapy, where e.g. monoclonal antibodies are administered to the individual in need thereof - monoclonal antibodies only recognize one single epitope, and they are not capable of triggering secondary immune responses. However, infusion of monoclonals has to be done during hospitalisation, and the amounts administered are in the order of grams of protein per treatment. The costs involved in treatment with monoclonal antibodies are thus a considerable concern.

Hence, there is definitely a need for agents that can invoke an effective immune response against specific epitopes in self-antigens without being subject to suppression due to immune reactivity with a carrier protein.

OBJECT OF THE INVENTION

It is an object of the present invention to provide novel immunogenic agents that are useful in induction of an immune response against a single epitope of an antigen of interest. It is a further object to provide for methods of use and preparation of such immunogenic agents as well as for various molecular biology tools useful in these methods.

SUMMARY OF THE INVENTION

It has been realised by the present inventor, that use of abundant self-proteins (such as autologous immunoglobulins) can provide for "scaffolds" or "carriers" that are able to display single 3D structures that correspond to or are identical to B-cell epitopes of antigens. Since such modified, abundant self-proteins are not normally capable of exhibiting sufficient immunogenicity so as to give rise to an effective immune response towards the B-cell epitope introduced in the scaffold structure, they are according to the invention engineered to also include foreign T helper epitopes that stimulate the necessary T-cell help for B-cells producing autoreactive antibodies.

Hence, in its broadest and most general aspect the present invention relates to a chimeric binding protein that is immunogenic in an animal, said chimeric binding protein being one that binds specifically to a first receptor, said first receptor being one that binds a second receptor present in an antigen of said animal, wherein said chimeric binding protein comprises:

- a B-cell epitope in the form of a binding site that specifically binds the first receptor and which competes with

the second receptor for binding to the first receptor,
- a scaffold protein structure that stabilises the 3D conformation of the binding site, said scaffold protein structure being autologous in said mammal, and
- at least one tolerance breaking amino acid sequence, which is heterologous in said animal and which binds to an MHC Class II molecule in said animal.

The present invention also relates to a nucleic acid fragment that encodes the chimeric protein of the present invention.

A third aspect of the present invention relates to a method of preparing the chimeric binding protein of the present invention.

Yet another aspect of the present invention relates to a vector that is capable of expressing the nucleic acid fragment of the invention.

A further aspect of the present invention relates to a method of immunizing against a self-antigen by administering an immunogenic agent of the invention so as to provoke an immune response against an epitope of said antigen.

LEGEND TO THE FIGURE

Fig. 1: Schematic depiction of a filamentous phage.
The figure shows a filamentous phage with its protein sheath that is made up of five different coating proteins (pIII, pVI, pVII, pVIII and pIX).

Fig. 2: Schematic depiction of phage display technology.
One round of panning consists of: Superinfection of the *E. coli* antibody library, production of recombinant phage

particles, incubation of phage with the antigen, removal of non-binding phage by a washing procedure, elution of bound phage particles and transduction of fresh *E. coli* cells with the eluted phage.

DETAILED DISCLOSURE OF THE INVENTION

Before detailing on the specifics of the present invention, a number of specific terms and expressions will be defined for the purposes of understanding of the present disclosure.

A "chimeric binding protein" is in the present context intended to mean a protein/polypeptide that includes a combination of amino acid sequences not found in association in the same molecule in nature. More specifically, the chimeric binding protein includes at least an amino acid sequence from a mammalian protein (the scaffold or carrier) and a foreign T_H epitope (foreign in the sense that it is not naturally associated with the remainder of the chimeric binding protein). Further, the term implies that the chimeric binding protein includes a structure that functions as a binding site. This binding site may be a natural part of the scaffold (in the case of an anti-idiotypic antibody) or it may be a binding site which is grafted onto or introduced into the scaffold.

The term "immunogen" in the present context refers to an agent (a substance or a composition of matter) that induces an immune response. It will be understood that certain molecules (*e.g.* traditional small haptens or self-proteins that are tolerated in the autologous host) are incapable of inducing an immune response.

The terms "T-lymphocyte" and "T-cell" will be used interchangeably for lymphocytes of thymic origin which are responsible for various cell mediated immune responses as well as for helper activity in the humeral immune response. Likewise, the terms "B-lymphocyte" and "B-cell" will be used interchangeably for antibody-producing lymphocytes.

The term "polypeptide" is in the present context intended to mean both short peptides of from 2 to 10 amino acid residues, oligopeptides of from 11 to 100 amino acid residues, and polypeptides of more than 100 amino acid residues. Furthermore, the term is also intended to include proteins, *i.e.* functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be covalently linked, or may be non-covalently linked. The polypeptide(s) in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups. Also, the term "polyamino acid" is an equivalent to the term "polypeptide".

The term "subsequence" means any consecutive stretch of at least 3 amino acids or, when relevant, of at least 3 nucleotides, derived directly from a naturally occurring amino acid sequence or nucleic acid sequence, respectively.

The term "animal" is in the present context in general intended to denote an animal species (preferably mammalian), such as *Homo sapiens*, *Canis domesticus*, etc. and not just one single animal. However, the term also denotes a population of such an animal species, since it is important that the individuals immunized according to the method of the invention all harbour substantially the same a allowing for immunization of the animals with the same immunogen(s). If, for instance, genetic variants of an antigen exist in different human subpopulations it may be necessary to use different immunogens in

these different populations in order to be able to break the autotolerance towards the antigen in each population. It will be clear to the skilled person that an animal in the present context is a living being which has an immune system. It is preferred that the animal is a vertebrate, such as a mammal.

By the term "*in vivo* down-regulation of antigen activity" is herein meant reduction in the living organism of the number of interactions between the antigen in question and its receptors/ligands or down-regulation of any other measurable physiological activity exerted by the antigen. The down-regulation can be obtained by means of several mechanisms: Of these, simple interference with the active site in an antigen by antibody binding is the most simple. However, it is also within the scope of the present invention that the antibody binding results in removal of antigen by scavenger cells (such as macrophages and other phagocytic cells). Another possibility is binding of antibodies to the antigen that are capable of interfering with the normal cleavage of a propeptide into a mature polypeptide.

The expression "effecting presentation ... to the immune system" is intended to denote that the animal's immune system is subjected to an immunogenic challenge in a controlled manner. As will appear from the disclosure below, such challenge of the immune system can be effected in a number of ways of which the most important are vaccination with polypeptide containing "pharmaccines" (*i.e.* a vaccine which is administered to treat or ameliorate ongoing disease) or nucleic acid "pharmaccine" vaccination, but also live and viral vaccines are within the scope of the present invention. The important result to achieve is that immune competent cells in the animal are confronted with the antigen in an immunologically effective manner, whereas the precise mode of achieving this result is

of less importance to the inventive idea underlying the present invention.

The term "immunogenically effective amount" has its usual meaning in the art of immunology, *i.e.* an amount of an immunogen which is capable of inducing an immune response which significantly engages molecules which share immunological features with the immunogen.

When using the expression that a protein has been "modified" is herein meant a chemical modification of the polypeptide which constitutes the backbone of the relevant protein that forms the scaffold part of a chimeric binding molecule of the present invention. Such a modification can *e.g.* be derivatization (*e.g.* alkylation, acylation, esterification etc.) of certain amino acid residues in the scaffold's polypeptide sequence, but as will be appreciated from the disclosure below, the preferred modifications comprise changes of (or additions to) the primary structure of the amino acid sequence.

When discussing "autotolerance towards" an antigen it is understood that since the antigens targeted by the present invention are self-antigens in the population to be vaccinated, normal individuals in the population do not mount an immune response against the antigen; it cannot be excluded, though, that occasional individuals in an animal population might be able to produce antibodies against the native antigen, *e.g.* as part of an autoimmune disorder. At any rate, an animal will normally only be autotolerant towards its own antigen, but it cannot be excluded that antigen analogues derived from other animal species or from a population having a different phenotype would also be tolerated by said animal. It is also important to remember that different levels of

tolerance exist. In the present invention, the animal's tolerance towards the scaffold protein structure is at a higher level than the tolerance towards the B-cell epitope part of the chimeric binding protein - in fact, the tolerance against the scaffold protein part is so high that no significant immunological effects are brought about by antibodies cross-reacting with the animal's naturally occurring scaffold protein.

A "foreign T-cell epitope" (or: "foreign T-lymphocyte epitope") is a peptide which is able to bind to an MHC molecule and which stimulates T-cells in an animal species. Preferred foreign T-cell epitopes in the invention are "promiscuous" epitopes, *i.e.* epitopes which bind to a substantial fraction of a particular class of MHC molecules in an animal species or population. Only a very limited number of such promiscuous T-cell epitopes are known, and they will be discussed in detail below. It should be noted that in order for the immunogens which are used according to the present invention to be effective in as large a fraction of an animal population as possible, it may be necessary to 1) insert several foreign T-cell epitopes in the same chimeric binding protein or 2) prepare several chimeric binding proteins wherein each has a different promiscuous epitope inserted. It should be noted also that the concept of foreign T-cell epitopes also encompasses use of cryptic T-cell epitopes, *i.e.* epitopes which are derived from a self-protein and which only exerts immunogenic behaviour when existing in isolated form without being part of the self-protein in question.

A "foreign T helper lymphocyte epitope" (a foreign T_H epitope) is a foreign T cell epitope which binds an MHC Class II molecule and can be presented on the surface of an antigen presenting cell (APC) bound to the MHC Class II molecule. In

this connection, the term "foreign" means that the T_H epitope is foreign to the immunized animal but also that the T_H epitope is not naturally associated with the scaffold protein structure that forms part of the chimeric binding protein of he invention. It will be understood that a "tolerance breaking amino acid sequence" includes at least one such foreign T_H epitope.

A "functional part" of a (bio)molecule is in the present context intended to mean the part of the molecule which is responsible for at least one of the biochemical or physiological effects exerted by the molecule. It is well-known in the art that many enzymes and other effector molecules have an active site which is responsible for the effects exerted by the molecule in question. Other parts of the molecule may serve a stabilizing or solubility enhancing purpose and can therefore be left out if these purposes are not of relevance in the context of a certain embodiment of the present invention. For instance it is possible to use certain other cytokines as a modifying moiety in the chimeric binding protein (cf. the detailed discussion below), and in such a case, the issue of stability may be irrelevant since the incorporation into the chimeric binding protein provides the stability necessary.

The term "adjuvant" has its usual meaning in the art of vaccine technology, *i.e.* a substance or a composition of matter which is 1) not in itself capable of mounting a specific immune response against the immunogen of the vaccine, but which is 2) nevertheless capable of enhancing the immune response against the immunogen. Or, in other words, vaccination with the adjuvant alone does not provide an immune response against the immunogen, vaccination with the immunogen may or may not give rise to an immune response against the immunogen, but the combination of vaccination with immunogen and adjuvant induces

an immune response against the immunogen which is stronger than that induced by the immunogen alone.

"Targeting" of a molecule is in the present context intended to denote the situation where a molecule upon introduction in the animal will appear preferentially in certain tissue(s) or will be preferentially associated with certain cells or cell types. The effect can be accomplished in a number of ways including formulation of the molecule in composition facilitating targeting or by introduction in the molecule of groups which facilitates targeting. These issues will be discussed in detail below.

"Stimulation of the immune system" means that a substance or composition of matter exhibits a general, non-specific immunostimulatory effect. A number of adjuvants and putative adjuvants (such as certain cytokines) share the ability to stimulate the immune system. The result of using an immunostimulating agent is an increased "alertness" of the immune system meaning that simultaneous or subsequent immunization with an immunogen induces a significantly more effective immune response compared to isolated use of the immunogen.

"Productive binding" means binding of a peptide to the MHC molecule (Class I or II) so as to be able to stimulate T-cells that engage a cell that present the peptide bound to the MHC molecule. For instance, a peptide bound to an MHC Class II molecule on the surface of an APC is said to be productively bound if this APC will stimulate a T_H cell that binds to the presented peptide-MHC Class II complex.

The present invention has been conceived based on the knowledge that a number of specific binding agents (monoclonal antibodies, soluble receptors, small molecule inhibitors etc.)

are accepted in the art of therapy as being safe and efficacious. Amongst these agents are Remicade® (Infliximab, an anti-TNF mAB), Herceptin® (Trastuzumab, an anti-HER-2 mAB), Xolair® (Omalizumab, an anti-IgE mAB), and Retuximab (an anti-CD20 mAB). However, most of these specific binding agents need to be administered frequently and under medical supervision and control in order to meet basic safety criteria. Hence, even though these molecules may lack patient compliance, they have proven to be safe and efficacious in clinical trials. It will be understood, based on the above, that the preferred antigens against which it is desired to immunize, are of human origin, *i.e.* that the B-cell epitope against which an immune response is directed is a B-cell epitope of a human antigen.

It has now been realised by the present inventor that it is possible to prepare vaccines that will induce polyclonal antibody responses against the very structures of the antigens that such art-recognized binding agents interact with - such an immune response will share the advantages of an administration of monoclonal antibodies (*i.a.* that secondary immune reactions are not triggered because only one antibody will bind the target molecule at a time) but will further have the advantage that the frequency of immunizations can be kept considerably lower than the frequency of administration of antibody. Another advantage is that the antibodies induced are polyclonal and therefore it will be possible to induce the strongest binding antibodies possible.

The technology is exercised by means of identification/isolation of 3D structures that precisely mimic (or are identical to) the structures that bind the clinical safe agent and incorporation into immunogenic variants of *abundant*, otherwise non-immunogenic self-proteins. In this sense the present invention discloses protein constructs that are specialised

variations of the immunogenic self-proteins of WO 95/05849 but an important difference is that the immune response against the self-protein part is insignificant, cf. below.

Without being limited to any theory, it is believed that the present chimeric binding proteins are effective in raising immune responses against single B-cell epitopes of self-antigens for the following reasons: When immunizing with e.g. an anti-idiotypic IgG antibody, it is observed that an immune response is generated against the B-cell epitope constituted by the idiotype of the anti-idiotypic antibody. However, if the remainder of the anti-idiotypic antibody is not autologous in the vaccinated animal, that part of the molecule will act as a traditional carrier protein, and hence a strong immune response will be generated against that part of the molecule. By using an anti-idiotypic antibody that is primarily autologous, this effect is effectively avoided on the expense, however, of an effective immune response against the idiotype structure - there is not sufficient stimulation of T_H lymphocytes to bring about a clinically significant B-cell response. The present inventor suggest in this particular setting to introduce strong foreign T_H epitope(s) in the anti-idiotypic antibody thereby breaking the tolerance towards the molecule.

Now, it could be suspected that an effective and even harmful immune response would be generated against the anti-idiotypic antibody as such, but it is here important to note that this autologous self-protein (IgG) is selected so as to be an abundant self-protein in the serum of the vaccinated individual. The immune system will therefore not be capable of raising a physiologically relevant response against the antibody, either because the immune system is completely incapable of raising a B-cell response against such proteins

due to B-cell anergy or, alternatively, antibody production is actually induced, but due to the abundance of the autologous antibody structure, the induced antibodies that do not react with the idiotype are "absorbed" due to binding to the large amounts of IgG in serum.

Therefore, irrespective of the underlying effect, the only relevant and effective immune response will be that directed against the idiotype of the anti-idiotypic antibody, since this part of the antibody response is not "diluted" by reactivity with the abundant serum protein.

It will be understood, that the present approach is not limited to the use of such immunogenized autologous anti-idiotypic antibodies. Other abundant self-proteins may serve as a carrier structure for the B-cell epitope it is of interest to immunize against - the importance is here that either no antibodies can be induced against the abundant self-protein, or, if antibodies are induced against the abundant self-protein, these should not be capable of exerting adverse effects due to their binding to the abundant self-protein *in vivo* - in other words, the concentration of the self-protein should be so high that it can absorb antibodies that cross-react with regions outside the B-cell epitope of interest.

Characteristics of the chimeric binding proteins of the invention.

The scaffold protein structure

The scaffold protein structure serves to purposes. First, the scaffold protein has to stabilise the native 3D conformation of the B-cell epitope against which the relevant immune response is intended to be raised. Second, when immunising

with the chimeric binding protein, the antibodies induced against the scaffold protein structure (if any are raised at all) should be incapable of giving rise to adverse effects.

With respect to the stabilising effect of the scaffold protein structure: It will normally be feasible to utilise an exposed loop structure or an active site in the scaffold protein structure. For instance, the complementarity determining regions (CDRs) of an antibody are kept in their correct conformation by parts of invariable parts of the heavy and light chains of an antibody, and the same holds true for active sites in enzymes and other molecules having active or binding sites.

With respect to the non-adverse nature of the scaffold protein structure, it is preferred to use such ones that are derived from abundant proteins, meaning that if any antibodies against such scaffold protein structures are produced, these antibodies will be absorbed by the large amounts of scaffold protein that is naturally occurring in the immunized individual. In this context, it is especially preferred that the scaffold protein structure is derived from an abundant serum protein.

The abundancy of such a serum protein must be at least so high that antibodies raised against the protein will not give rise to any adverse immunological effects. Thus, according to the invention, the normal serum concentration of an abundant serum protein is at least 0.1 g per 100 ml serum. It is even more preferred that the normal serum concentration of the abundant serum protein is at least 0.3 g per 100 ml serum, such as at least 0.4, at least 0.6, at least 0.8, at least 1.0, at least 1.2, at least 1.4, and at least 1.5 g per 100 ml serum. Even higher values are preferred when the abundant serum protein is

albumin: at least 2.0 g per 100 ml, at least 2.5, at least 3.0, at least 3.5, and at least 4.0 g per 100 ml serum.

Hence, especially interesting scaffold protein structures are derived from albumin, an immunoglobulin (such as IgG and IgA), transferrin, α2-macroglobulin, haptaglobin, α_1-lipoprotein, β_1-lipoprotein, all of which are proteins having a high serum concentration.

It is particularly preferred that the chimeric binding protein is derived from an antibody molecule such as IgG. The scaffold protein structure will then typically be derived from the non-idiotypic region of the molecule, *i.a.* from the non-idiotypic region of a complete antibody or from a fragment thereof such as an F(ab')2 fragment, an Fab fragment, and an scFv. By using a complete antibody or at least a fragment that contains a large part of the Fc part of the antibody, it is attained that the chimeric binding protein includes a self-adjuvating component.

Hence, in preferred embodiments, the scaffold protein structure comprises a substantially complete amino acid sequence of a polypeptide autologous in said animal, e.g. a substantially complete amino acid sequence of the Fc of a heavy and/or light chain from an antibody of the animal. Further, in order for the scaffold protein structure to immunologically resemble its parent autologous protein, it is preferred that said scaffold protein structure comprises a substantial number of B-cell epitopes found in the autologous scaffold protein structure in the animal. It is most preferred that the scaffold protein structure has substantially the same tertiary (and, if relevant, quaternary) structure of a polypeptide autologous in said animal. Again, if the scaffold protein structure is derived from an antibody, this will

entail that the chimeric binding protein includes a substantially complete antibody molecule within its structure.

Methods for preserving tertiary structure and also quaternary structure of autologous proteins are known in the art. For instance, it is possible to prepare analogous self-proteins where foreign T_H epitopes have been introduced, cf. e.g. WO 95/05849, WO 00/20027, and WO 00/15807 - these teaching can, according to the present invention be utilised with respect to modifications made on the scaffold protein structure that forms part of the chimeric binding proteins of the present invention.

Also methods for preserving quaternary structure are described in the art and can be readily applied to suitable scaffold protein structures. One possibility is to prepare multimeric proteins where only one of the monomeric units involve a modification according to the invention (*i.e.* a monomeric unit that has been modified by preserving the tertiary structure of this unit so as to allow the monomer to interact correctly with native monomeric units of the multimeric proteins) and it is even possible to prepare variants of multimers wherein each of the monomeric units have been modified according to these principles. However, yet another possibility is to "monomerize" otherwise multimeric proteins by following teachings in PCT/DK02/00764 (which is hereby incorporated by reference). Also other teachings of this reference that relate to suitable modifications of monomeric units that are sufficiently structure preserving so as to allow multimerization are relevant for the purposes of the present invention.

In the event the scaffold protein structure is an IgG molecule, it is sometimes advantageous to use a sub-class that is

unable to fix complement, so as to completely avoid secondary immune mechanisms and their actions. This is e.g. of interest when the target for immunization with the chimeric binding protein is IgE. However, it is believed that this precaution is of less relevance, since the clinically relevant antibodies induced by the chimeric binding proteins of the present invention will never bind simultaneously to the same antigen (unless the epitope is of repetitive nature as in certain carbohydrates) - only one single epitope is bound. Since complement fixation and activation requires binding of more than one antibody, this and other secondary effector mechanisms are not believed to be an issue.

The B-cell epitope part of the chimeric binding protein

As will be understood from the above, the scaffold protein structure serves the purpose of presenting the B-cell epitope of interest in a spatial conformation that is quasi-identical or identical to an antigenic determinant in an antigen found in the animal to be vaccinated.

One way of achieving this goal is to graft an identified B-cell epitope of an antigen into a known presentation structure or scaffold. For instance, it is known in the art to prepare genetically modified antibodies where the complementarity determining regions (CDRs) of antibodies are modified to present the amino acid sequence of a known antigenic epitope.

Alternatively, the B-cell epitope may be any amino acid sequence that can be isolated according to the methods of the invention, cf. below. As discussed above, the invention relies in part on the use of anti-idiotypic antibodies, where the idiotype constitutes the B-cell epitope part of the chimeric binding protein, but other abundant serum proteins can be

randomly modified in an exposed loop structure and screened according to the invention so as to isolate suitable binding species. By utilising the inventive screening methods of the invention, it is ensured that the B-cell epitope part of the chimeric binding protein is indeed presented in a suitable conformation, and it is hence not necessary to rely on introduction of the B-cell epitope in a scaffold that may or may not be capable of presenting the B-cell epitope in a satisfactory manner.

So, the chimeric binding protein of the invention preferably contains the B-cell epitope which is constituted by the idiotype of an antibody. This has several reasons: First of all, an idiotype of an antibody has a defined 3D conformation that is known to bind to an epitope. Further, if the idiotype of the antibody is reactive with a different antibody's idiotype, then the antibody's idiotype will mimic the antigenic epitope against which the different antibody reacts. Hence, there is no need to test whether or not the epitope in question will be presented sufficiently well in a chosen scaffold protein structure, and it does not matter whether the epitope is regional or assembled topographic in nature. Furthermore, anti-idiotypic antibodies have already been demonstrated to be capable of functioning as vaccination agents so as to raise an immune response against the idiotype. Finally, by using this strategy, it is possible to device completely proteinaceous vaccine agents that can elicit antibody responses against non-proteinaceous antigens.

Therefore, in preferred embodiments of the present invention, the chimeric binding protein of the invention is one wherein said first receptor is the idiotype of an antibody or a specific binding region of a ligand that binds the second receptor in the animal to be immunized. In this context, it is

especially preferred that the chimeric binding protein of the invention is one, wherein said first receptor is the idiotype of a monoclonal antibody.

The tolerance breaking amino acid sequence and its position

Of course, the tolerance breaking amino acid sequence may be situated in any suitable position in the chimeric binding protein. For instance, it may be introduced in the form of a simple fusion partner or conjugation partner to the scaffold protein structure and in these embodiments the tolerance breaking amino acid sequence may constitute a complete protein. Technologies relevant for this are well-known in the art.

However, it is preferred that the tolerance breaking amino acid sequence is introduced by means of amino acid insertion or substitution in the amino acid sequence of the scaffold protein structure. An especially preferred chimeric binding protein of the invention is an anti-idiotypic antibody or an effectively binding fragment thereof that is modified so as to include said tolerance breaking amino acid sequence, where said anti-idiotypic antibody is autologous in the animal to be immunized.

As mentioned above, the introduction of an tolerance breaking amino acid sequence can be accomplished by introduction of at least one amino acid insertion, addition, deletion, or substitution in the scaffold protein structure. Of course, the normal situation will be the introduction of more than one change in the amino acid sequence (*e.g.* insertion of or substitution by a complete T-cell epitope) but the important goal to reach is that the chimeric binding protein, when processed by an antigen presenting cell (APC), will give rise

to a tolerance breaking amino acid sequence being presented in context of an MCH Class II molecule on the surface of the APC. Thus, if the amino acid sequence of the scaffold protein structure in appropriate positions comprises a number of amino acid residues which can also be found in a tolerance breaking amino acid sequence, then the introduction of such a tolerance breaking amino acid sequence can be accomplished by providing the remaining amino acids of the foreign epitope by means of amino acid insertion, addition, deletion and substitution. In other words, it is not necessary to introduce a complete T_H epitope by insertion or substitution in order to fulfil the purpose of the present invention.

It is preferred that the number of amino acid insertions, deletions, substitutions or additions is at least 2, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 25 insertions, substitutions, additions or deletions. It is furthermore preferred that the number of amino acid insertions, substitutions, additions or deletions is not in excess of 150, such as at most 100, at most 90, at most 80, and at most 70. It is especially preferred that the number of substitutions, insertions, deletions, or additions does not exceed 60, and in particular the number should not exceed 50 or even 40. Most preferred is a number of not more than 30. With respect to amino acid additions, it should be noted that these, when the resulting construct is in the form of a fusion polypeptide, is often considerably higher than 150.

Preferred embodiments of the invention include introducing at least one *immunodominant* tolerance breaking amino acid sequence (in this specification also denoted "a foreign immune dominant T_H epitope"). It will be understood that the question of immune dominance of a T_H epitope depends on the animal

species in question. As used herein, the term "immunodominance" simply refers to epitopes which in the vaccinated individual/population gives rise to a significant immune response, but it is a well-known fact that a T_H epitope which is immunodominant in one individual/population is not necessarily immunodominant in another individual of the same species, even though it may be capable of binding MHC-II molecules in the latter individual. Hence, for the purposes of the present invention, an immune dominant T_H epitope is a T_H epitope which will be effective in providing T-cell help when present in an antigen. Typically, immune dominant T_H epitopes have as an inherent feature that they will substantially always be presented bound to an MHC Class II molecule, irrespective of the polypeptide wherein they appear.

Another important point is the issue of MHC restriction of T_H epitopes. In general, naturally occurring T_H epitopes are MHC restricted, *i.e.* a certain peptide constituting a T_H epitope will only bind effectively to a subset of MHC Class II molecules. This in turn has the effect that in most cases the use of one specific T_H epitope will result in a vaccine component which is only effective in a fraction of the population, and depending on the size of that fraction, it can be necessary to include more T_H epitopes in the same molecule, or alternatively prepare a multi-component vaccine wherein the components are variants chimeric binding protein which are distinguished from each other by the nature of the T_H epitope introduced.

If the MHC restriction of the T_H cells used is completely unknown (for instance in a situation where the vaccinated animal has a poorly defined MHC composition), the fraction of the population covered by a specific vaccine composition can be determined by means of the following formula

(II) $$f_{population} = 1 - \prod_{i=1}^{n} (1 - p_i)$$

-where p_i is the frequency in the population of responders to the i^{th} foreign T_H epitope present in the vaccine composition, and n is the total number of foreign T_H epitopes in the vaccine composition. Thus, a vaccine composition containing 3 foreign T_H epitopes having response frequencies in the population of 0.8, 0.7, and 0.6, respectively, would give

$$1 - 0.2 \times 0.3 \times 0.4 = 0.976$$

-*i.e.* 97.6 percent of the population will statistically mount an MHC-II mediated response to the vaccine.

The above formula does not apply in situations where a more or less precise MHC restriction pattern of the peptides used is known. If, for instance a certain peptide only binds the human MHC-II molecules encoded by HLA-DR alleles DR1, DR3, DR5, and DR7, then the use of this peptide together with another peptide which binds the remaining MHC-II molecules encoded by HLA-DR alleles will accomplish 100% coverage in the population in question. Likewise, if the second peptide only binds DR3 and DR5, the addition of this peptide will not increase the coverage at all. If one bases the calculation of population response purely on MHC restriction of T_H epitopes in the vaccine, the fraction of the population covered by a specific vaccine composition can be determined by means of the following formula:

(III) $$f_{population} = 1 - \prod_{j=1}^{3} (1 - \varphi_j)^2$$

-wherein φ_j is the sum of frequencies in the population of allelic haplotypes encoding MHC molecules which bind any one of the T_H epitopes in the vaccine and which belong to the j^{th}

of the 3 known HLA loci (DP, DR and DQ); in practice, it is first determined which MHC molecules will recognize each T_H epitope in the vaccine and thereafter these are listed by type (DP, DR and DQ) - then, the individual frequencies of the different listed allelic haplotypes are summed for each type, thereby yielding φ_1, φ_2, and φ_3.

It may occur that the value p_i in formula II exceeds the corresponding theoretical value π_i:

(IV) $$\pi_i = 1 - \prod_{j=1}^{3}(1-\nu_j)^2$$

-wherein υ_j is the sum of frequencies in the population of allelic haplotype encoding MHC molecules which bind the i^{th} T_H epitope in the vaccine and which belong to the j^{th} of the 3 known HLA loci (DP, DR and DQ). This means that in $1-\pi_i$ of the population is a frequency of responders of $f_{residual\_i} = (p_i - \pi_i)/(1-\pi_i)$. Therefore, formula III can be adjusted so as to yield formula V:

(V) $$f_{population} = 1 - \prod_{j=1}^{3}(1-\varphi_j)^2 + \left(1 - \prod_{i=1}^{n}(1-f_{residual\_i})\right)$$

-where the term $1-f_{residual-i}$ is set to zero if negative. It should be noted that formula V requires that all epitopes have been haplotype mapped against identical sets of haplotypes.

Therefore, when selecting tolerance breaking amino acid sequences to be introduced in the chimeric binding protein, it is important to include all knowledge of the epitopes which is available: 1) The frequency of responders in the population to each epitope, 2) MHC restriction data, and 3) frequency in the population of the relevant haplotypes.

There exist a number of naturally occurring "promiscuous" T_H epitopes which are active in a large proportion of individuals

of an animal species or an animal population and these are preferably introduced in the vaccine thereby reducing the need for a very large number of different analogues in the same vaccine.

The promiscuous epitope can according to the invention be a naturally occurring human T_H epitope such as epitopes from tetanus toxoid (*e.g.* the P2 and P30 epitopes), diphtheria toxoid, Influenza virus hemagluttinin (HA), and *P. falciparum* CS antigen. The sequences of the P2 and P30 epitopes are Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu-Leu and Phe-Asn-Asn-Phe-Thr-Val-Ser-Phe-Trp-Leu-Arg-Val-Pro-Lys-Val-Ser-Ala-Ser-His-Leu-Glu, respetively.

Over the years a number of other promiscuous T_H epitopes have been identified. Especially peptides capable of binding a large proportion of HLA-DR molecules encoded by the different HLA-DR alleles have been identified and these are all possible T-cell epitopes to be introduced in the analogues used according to the present invention. Cf. also the epitopes discussed in the following references which are hereby all incorporated by reference herein: WO 98/23635 (Frazer IH et al., assigned to The University of Queensland); Southwood S *et al.*, 1998, J. Immunol. **160**: 3363-3373; Sinigaglia F *et al.*, 1988, Nature **336**: 778-780; Chicz RM *et al.*, 1993, J. Exp. Med **178**: 27-47; Hammer J *et al.*, 1993, Cell **74**: 197-203; and Falk K *et al.*, 1994, Immunogenetics **39**: 230-242. The latter reference also deals with HLA-DQ and -DP ligands. All epitopes listed in these references are relevant as candidate tolerance breaking amino acid sequences to be used in the present invention, as are epitopes which share common motifs with these.

Alternatively, the tolerance breaking amino acid sequence can be any artificial T_H epitope, which is capable of binding a

large proportion of MHC Class II molecules. In this context the pan DR epitope peptides ("PADRE") described in WO 95/07707 and in the corresponding paper Alexander J *et al.*, 1994, Immunity **1**: 751-761 (both disclosures are incorporated by reference herein) are interesting candidates for tolerance breaking amino acid sequences to be used according to the present invention. It should be noted that the most effective PADRE peptides disclosed in these papers carry D-amino acids in the C- and N-termini in order to improve stability when administered. However, the present invention primarily aims at incorporating the relevant epitopes as part of the chimeric binding protein, which should then subsequently be broken down enzymatically inside the lysosomal compartment of APCs to allow subsequent presentation in the context of an MHC-II molecule and therefore it is not expedient to incorporate D-amino acids in the epitopes used in the present invention.

One especially preferred PADRE peptide is the one having the amino acid sequence AKFVAAWTLKAAA or an immunologically effective subsequence thereof. This, and other epitopes having the same lack of MHC restriction are preferred tolerance breaking amino acid sequences which should be present in the chimeric binding proteins of the invention. Such super-promiscuous epitopes will allow for the simplest embodiments of the invention wherein only one single chimeric binding protein species is presented to the vaccinated animal's immune system.

It is understood from the above, that the introduction of a tolerance breaking amino acid sequence in the scaffold protein structure should result in preservation of a substantial fraction of B-cell epitopes or even the 3D structure of the autologous scaffold protein (or domains thereof) which is subjected to modification as described herein. Confirmation of this preservation can be confirmed in several ways. One is

simply to prepare a polyclonal antiserum directed against the native molecule serving as a scaffold (e.g. an antiserum prepared in a rabbit or another suitable animal) and thereafter use this antiserum as a test reagent (e.g. in a competitive ELISA) against the chimeric binding proteins which are produced. Chimeric binding proteins which react to the same extent with the antiserum as does the autologous scaffold protein structure must be regarded as having the same 3D structure as the autologous scaffold protein structure whereas chimeric molecules exhibiting a limited (but still significant and specific) reactivity with such an antiserum is regarded as having maintained a substantial fraction of the original B-cell epitopes.

Alternatively, a selection of monoclonal antibodies reactive with distinct epitopes on the autologous scaffold protein can be prepared and used as a test panel. This approach has the advantage of allowing 1) an epitope mapping of the autologous scaffold molecule and 2) a mapping of the epitopes which are maintained in the chimeric binding protein.

Of course, a third approach would be resolve the 3-dimensional structure of the autologous scaffold protein or of a biologically active truncate thereof (cf. above) and compare this to the resolved three-dimensional structure of the chimeric binding protein. Three-dimensional structure can be resolved by the aid of X-ray diffraction studies and NMR-spectroscopy. Further information relating to the tertiary structure can to some extent be obtained from circular dichroism studies which have the advantage of merely requiring the polypeptide in pure form (whereas X-ray diffraction requires the provision of crystallized polypeptide and NMR requires the provision of isotopic variants of the polypeptide and also involves the problem of a size restriction on the polypeptides to resolve)

in order to provide useful information about the tertiary structure of a given molecule. However, ultimately X-ray diffraction and/or NMR are necessary to obtain conclusive data since circular dichroism can only provide indirect evidence of correct 3-dimensional structure via information of secondary structure elements.

The present invention relies on the identification of especially well-suited regions for introduction in the scaffold protein structure of the foreign element that must provide for the necessary T_H epitopes. Especially preferred regions are flexible loop regions (which do not contribute directly to tertiary structure) as well as flexible linker regions and N or C termini. Alternatively, the introduction of the T_H epitope can be made in a region that has a secondary structure that has a high degree of similarity with the secondary structure of the epitope (an α-helical region may be substituted with an epitope that can form part of an α-helix, a β-strand region may be substituted with an epitope that can form part of a β-strand region).

It is important to note that when a chimeric binding protein is prepared by amino acid substitution in the scaffold protein structure with a tolerance breaking amino acid sequence, the introduction is supposed to influence minimally on the epitopes in the relevant scaffold protein. Hence, normally a substitution will only result in a variant where the deleted amino acids constitute 30% or less of the relevant scaffolde protein (sub)sequence, and under normal circumstances this number will be much lower such as at most 20%, at most 15%, at most 10%, and at most 7.5%. Being a large molecule such as a complete antibody, the number can be even lower, such as at

most 5%, at most 4% and even as little as at most 3% or at most 2%.

Optional constituents of the chimeric binding protein

Apart from including the above-discussed B-cell epitope in the form of a binding site, the scaffold protein structure and the tolerance breaking amino acid sequence, the chimeric binding protein of the invention may also include a number of other features. In these embodiments, the chimeric binding protein includes

- at least one first moiety that effects targeting of the chimeric binding protein to an antigen presenting cell (APC), and/or
- at least one second moiety that stimulates the immune system, and/or
- at least one third moiety that optimises presentation of the chimeric binding protein to the immune system.

The functional and structural features relating these first, second and third moieties will be discussed in the following:

They can be present in the form of side groups attached covalently or non-covalently to suitable chemical groups in the amino acid sequence of the scaffold protein structure. This is to mean that stretches of amino acid residues derived from the autologous scaffold protein are derivatized without altering the primary amino acid sequence, or at least without introducing changes in the peptide bonds between the individual amino acids in the chain.

The moieties can also be in the form of fusion partners to the amino acid sequence derived from the autologous scaffold protein and/or the tolerance breaking amino acid sequence. In

this connection it should be mentioned that both possibilities include the option of conjugating the amino acid sequence to a carrier, cf. the discussion of these below. In other words, in the present context the term "fusion protein" is not merely restricted to a fusion construct prepared by means of expression of a DNA fragment encoding the construct but also to a conjugate between two proteins which are joined by means of a peptide bond in a subsequent chemical reaction.

As mentioned above, the chimeric binding protein can also include the introduction of a first moiety which targets the chimeric binding protein to an APC or a B-lymphocyte. For instance, the first moiety can be a specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen. Many such specific surface antigens are known in the art. For instance, the moiety can be a carbohydrate for which there is a receptor on the B-lymphocyte or the APC (e.g. mannan or mannose). Alternatively, the second moiety can be a hapten. Also an antibody fragment which specifically recognizes a surface molecule on APCs or lymphocytes can be used as a first moiety (the surface molecule can e.g. be an FCγ receptor of macrophages and monocytes, such as FCγRI or, alternatively any other specific surface marker such as CD40 or CTLA-4) - in this context it is noteworthy that use of chimeric binding proteins of the invention that are anti-idiotypic IgG provides for such a first moiety in the form of the Fcγ receptor binding region of IgG.

It should be noted that all these exemplary targeting molecules can be used as part of an adjuvant, cf. below. CD40 ligand, antibodies against CD40, or variants thereof which bind CD40 will target the chimeric binding protein to

dendritic cells. At the same time, recent results have shown that the interaction with the CD40 molecule renders the T_H cells unessential for obtaining a CTL response. Hence, it is contemplated that the general use of CD40 binding molecules as the first moiety (or as adjuvants, cf. below) will enhance the CTL response considerably; in fact, the use of such CD40 binding molecules as adjuvants and "first moieties" in the meaning of the present invention is believed to be inventive in its own right.

As an alternative or supplement to targeting the chimeric binding protein to a certain cell type in order to achieve an enhanced immune response, it is possible to increase the level of responsiveness of the immune system by including the above-mentioned second moiety which stimulates the immune system. Typical examples of such second moieties are cytokines, heat-shock proteins, and hormones, as well as effective parts thereof.

Suitable cytokines to be used according to the invention are those which will normally also function as adjuvants in a vaccine composition, e.g. interferon γ (IFN-γ), Flt3 ligand (Flt3L), interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF); alternatively, the functional part of the cytokine molecule may suffice as the second moiety. With respect to the use of such cytokines as adjuvant substances, cf. the discussion below.

Alternatively, the second moiety can be a toxin, such as listeriolycin (LLO), lipid A and heat-labile enterotoxin. Also, a number of mycobacterial derivatives such as MDP (muramyl

dipeptide), CFA (complete Freund's adjuvant) and the trehalose diesters TDM and TDE are interesting possibilities.

According to the invention, suitable heat shock proteins used as the second moiety can be HSP70, HSP90, HSC70 (a heat shock cognate), GRP94, and calreticulin (CRT).

Also the possibility of introducing a third moiety that enhances the presentation of the chimeric binding protein to the immune system is an important embodiment of the invention. The art has shown several examples of this principle. For instance, it is known that the palmitoyl lipidation anchor in the *Borrelia burgdorferi* protein OspA can be utilised so as to provide self-adjuvating polypeptides (cf. e.g. WO 96/40718). It seems that the lipidated proteins form up micelle-like structures with a core consisting of the lipidation anchor parts of the polypeptides and the remaining parts of the molecule protruding therefrom, resulting in multiple presentations of the antigenic determinants. Hence, the use of this and related approaches using different lipidation anchors (e.g. a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group) are preferred embodiments of the invention, especially since the provision of such a lipidation anchor in a recombinantly produced protein is fairly straightforward and merely requires use of e.g. a naturally occurring signal sequence as a fusion partner for the chimeric binding protein, cf. below. Another possibility is use of the C3d fragment of complement factor C3 or C3 itself (cf. Dempsey et al., 1996, Science 271, 348-350 and Lou & Kohler, 1998, Nature Biotechnology 16, 458-462).

Targets for immunization according to the invention

The chimeric binding proteins of the present invention are especially suited for active specific immunotherapy that targets antigens that already are known as suitable targets for monoclonal antibody therapy. For instance, immunoglobulin E, CD20, CD11a, beta amyloid, HER-2, and TNFα are all suitable targets for the present invention, meaning that the above-discussed second receptor is present in one of these antigens. However, it is desirable to utilise the chimeric binding proteins in the active specific immunotherapy that targets any other proteinaceous or non-proteinaceous antigen where it is sufficient (or even necessary) to target one single epitope.

Such targets can be ones where it is desirable to only target a very specific "safe" region of the molecule (as in the case of IgE, where it is desirable to target the FCεR binding region to avoid problems with anaphylaxis induced by cross-linking anti-IgE antibodies), or it may be a defined exposed region of an antigen which is otherwise not accessible to antibodies (as with CD20, where only a minor portion is exposed to the extracellular phase.

However, in all cases where monoclonal antibody therapy has proven successful or where therapy with other ligands (such as soluble receptors) that bind a specific, therapeutically safe epitope or receptor structure, it is of value to prepare chimeric binding agents according to the present invention.

Nucleic acids and other molecular biology tools

The invention also relates to a nucleic acid fragment that encodes a chimeric binding protein of the invention. Such nucleic acid fragments are useful for recombinant preparation

of the chimeric binding proteins of the invention but also as parts of immunizing agents for nucleic acid vaccination, cf. below.

It will be appreciated from the above disclosure that the chimeric binding proteins can be prepared by means of recombinant gene technology but also by means of chemical synthesis or semisynthesis; the latter two options are especially relevant when the modification consists in coupling to protein carriers (such as KLH, diphtheria toxoid, tetanus toxoid, and BSA) and non-proteinaceous molecules such as carbohydrate polymers and of course also when the modification comprises addition of side chains or side groups to an polypeptide-derived peptide chain.

For the purpose of recombinant gene technology, and of course also for the purpose of nucleic acid immunization, nucleic acid fragments encoding the chimeric binding proteins or polypeptides are important chemical products. Hence, an important part of the invention pertains to a nucleic acid fragment which encodes chimeric binging protein described above, preferably a polypeptide wherein has been introduced a foreign T_H-cell epitope in the scaffold protein structure by means of insertion and/or addition, preferably by means of substitution and/or deletion. The nucleic acid fragments of the invention are either DNA or RNA fragments. Also included within the scope of the present invention are nucleic acid fragments complementary to a nucleic acid fragment encoding a chimeric binding protein of the invention.

The nucleic acid fragments of the invention will normally be inserted in suitable vectors to form cloning or expression vectors carrying the nucleic acid fragments of the invention; such novel vectors are also part of the invention. Details

concerning the construction of these vectors of the invention will be discussed in context of transformed cells and microorganisms below. The vectors can, depending on purpose and type of application, be in the form of plasmids, phages, cosmids, mini-chromosomes, or virus, but also naked DNA which is only expressed transiently in certain cells is an important vector. Preferred cloning and expression vectors of the invention are capable of autonomous replication, thereby enabling high copy-numbers for the purposes of high-level expression or high-level replication for subsequent cloning.

The general outline of a vector of the invention comprises the following features in the 5'→3' direction and in operable linkage: a promoter for driving expression of the nucleic acid fragment of the invention, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment of the invention, and a nucleic acid sequence encoding a terminator. When operating with expression vectors in producer strains or cell-lines it is for the purposes of genetic stability of the transformed cell preferred that the vector when introduced into a host cell is integrated in the host cell genome. In contrast, when working with vectors to be used for effecting *in vivo* expression in an animal (i.e. when using the vector in DNA vaccination) it is for security reasons preferred that the vector is not capable of being integrated in the host cell genome; typically, naked DNA or non-integrating viral vectors are used, the choices of which are well-known to the person skilled in the art.

The vectors of the invention are used to transform host cells to produce the chimeric binding protein of the invention. Such transformed cells, which are also part of the invention, can be cultured cells or cell lines used for propagation of the

nucleic acid fragments and vectors of the invention, or used for recombinant production of the chimeric binding protein of the invention. Alternatively, the transformed cells can be suitable live vaccine strains wherein the nucleic acid fragment (one single or multiple copies) have been inserted so as to effect secretion or integration into the bacterial membrane or cell-wall of the chimeric binding protein.

Preferred transformed cells of the invention are microorganisms such as bacteria (such as the species Escherichia [e.g. *E. coli*], Bacillus [e.g. Bacillus subtilis], Salmonella, or Mycobacterium [preferably non-pathogenic, e.g. M. bovis BCG]), yeasts (such as Saccharomyces cerevisiae), and protozoans. Alternatively, the transformed cells are derived from a multicellular organism such as a fungus, an insect cell, a plant cell, or a mammalian cell. Most preferred are cells derived from a human being, cf. the discussion of cell lines and vectors below.

For the purposes of cloning and/or optimised expression it is preferred that the transformed cell is capable of replicating the nucleic acid fragment of the invention. Cells expressing the nucleic fragment are preferred useful embodiments of the invention; they can be used for small-scale or large-scale preparation of the chimeric binding proteins or, in the case of non-pathogenic bacteria, as vaccine constituents in a live vaccine.

When producing the chimeric binding proteins of the invention by means of transformed cells, it is convenient, although far from essential, that the expression product is either exported out into the culture medium or carried on the surface of the transformed cell.

When an effective producer cell has been identified it is preferred, on the basis thereof, to establish a stable cell line which carries the vector of the invention and which expresses the nucleic acid fragment encoding the chimeric binding protein. Preferably, this stable cell line secretes or carries the chimeric binding protein of the invention, thereby facilitating purification thereof.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with the hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (see, e.g., Bolivar et al., 1977). The pBR322 plasmid contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the prokaryotic microorganism for expression.

Those promoters most commonly used in recombinant DNA construction include the B-lactamase (penicillinase) and lactose promoter systems (Chang et al., 1978; Itakura et al., 1977; Goeddel et al., 1979) and a tryptophan (trp) promoter system (Goeddel et al., 1979; EP-A-0 036 776). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebwenlist et al., 1980). Certain genes from prokaryotes may be expressed efficiently in *E. coli* from their own promoter sequences, pre-

cluding the need for addition of another promoter by artificial means.

In addition to prokaryotes, eukaryotic microbes, such as yeast cultures may also be used, and here the promoter should be capable of driving expression. *Saccharomyces cerevisiase*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available such as *Pichia pastoris*. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb et al., 1979; Kingsman et al., 1979; Tschemper et al., 1980). This plasmid already contains the trpl gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzman et al., 1980) or other glycolytic enzymes (Hess et al., 1968; Holland et al., 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination.

Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter re-

gion for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing a yeast-compatible promoter, origin of replication and termination sequences is suitable.

In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However, interest has been greatest in vertebrate cells, and propagation of vertebrate in culture (tissue culture) has become a routine procedure in recent years (Tissue Culture, 1973). Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 293 and MDCK cell lines.

Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., 1978). Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250

bp sequence extending from the *Hind*III site toward the *Bgl*I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g., Polyoma, Adeno, VSV, BPV) or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

Compositions of the invention

As will be apparent from the above, it is of interest to prepare immunogenic compositions that include chimeric binding proteins of the invention, nucleic acid fragments of the invention or non-pathogenic virus or bacteria that are capable of functioning as immunogenic agents because they express the chimeric binding proteins of the invention.

Thus, the invention also relates to a composition for inducing production of antibodies against an antigen in the autologous host, the composition comprising

- a chimeric binding proteinor the invention or a nucleic acid fragment of the invention or a vector of the invention (including such vectors as non-pathogenic virus or microorganisms, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant

Discussion of immunologically acceptable carriers and adjuvants follow in the sections regarding vaccination technologies of the invention.

Polypeptide vaccination

The invention relates to a method for down-regulating a self-antigen or a cell that displays epitopes of said self-antigen in an animal, the method comprising presenting the animal's immune system with an immunogenically effective amount of a chimeric binding protein of the invention. One preferred way of achieving this goal entails administration to the animal in question of an immunogenically effective amount of the chimeric binding protein. Preferably, the chimeric binding protein is formulated together with a pharmaceutically and immunologically acceptable carrier and/or vehicle and, optionally an adjuvant.

When effecting presentation of the chimeric binding protein to an animal's immune system by means of administration thereof to the animal, the formulation of the polypeptide follows the principles generally acknowledged in the art.

Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by US Patents 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all incorporated herein by reference. Typically, such vaccines are prepared as injectables either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are,

for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccines; cf. the detailed discussion of adjuvants below.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously, intracutaneously, or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral, buccal, sublinqual, intraperitoneal, intravaginal, anal and intracranial formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of active ingredient, preferably 25-70%. For oral formulations, cholera toxin is an interesting formulation partner (and also a possible conjugation partner).

The chimeric binding protein may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may

also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1 µg to 2000 µg (even though higher amounts in the 1-10 mg range are contemplated), such as in the range from about 0.5 µg to 1000 µg, preferably in the range from 1 µg to 500 µg and especially in the range from about 10 µg to 100 µg. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.

The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the age of the person to be vaccinated and the formulation of the antigen.

Some of the chimeric binding proteins of the vaccine are sufficiently immunogenic in a vaccine, but for some of the others the immune response will be enhanced if the vaccine further comprises an adjuvant substance. It is especially

preferred to use an adjuvant which can be demonstrated to facilitate breaking of the autotolerance to autoantigens.

Various methods of achieving adjuvant effect for the vaccine are known. General principles and methods are detailed in "The Theory and Practical Application of Adjuvants", 1995, Duncan E.S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-95170-6, and also in "Vaccines: New Generation Immunological Adjuvants", 1995, Gregoriadis G et al. (eds.), Plenum Press, New York, ISBN 0-306-45283-9, both of which are hereby incorporated by reference herein.

One group of preferred adjuvants facilitate uptake of the vaccine molecules by APCs, such as dendritic cells, and activate these. Non-limiting examples are selected from the group consisting of an immune targeting adjuvant; an immune modulating adjuvant such as a toxin, a cytokine, and a mycobacterial derivative; an oil formulation; a polymer; a micelle forming adjuvant; a saponin; an immunostimulating complex matrix (ISCOM matrix); a particle; DDA; aluminium adjuvants; DNA adjuvants; γ-inulin; and an encapsulating adjuvant. In general it should be noted that the disclosures above which relate to compounds and agents useful as first, second and third moieties in the chimeric binding protein also refer *mutatis mutandis* to their use in the adjuvant of a vaccine of the invention.

The application of adjuvants include use of agents such as aluminium hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent solution in buffered saline, admixture with synthetic polymers of sugars (e.g. Carbopol®) used as 0.25 percent solution, aggregation of the protein in the vaccine by heat treatment with temperatures ranging between 70° to 101°C for 30 second to 2 minute periods respectively and also aggre-

gation by means of cross-linking agents are possible. Aggregation by reactivation with pepsin treated antibodies (Fab fragments) to albumin, mixture with bacterial cells such as C. parvum or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed. Admixture with oils such as squalene and IFA is also preferred.

According to the invention DDA (dimethyldioctadecylammonium bromide) is an interesting candidate for an adjuvant as is DNA, MF59, and γ-inulin, but also Freund's complete and incomplete adjuvants as well as quillaja saponins such as QuilA and QS21 are interesting. Further possibilities are monophosphoryl lipid A (MPL), and the above mentioned C3 and C3d.

Liposome formulations are also known to confer adjuvant effects, and therefore liposome adjuvants are preferred according to the invention.

Also immunostimulating complex matrix type (ISCOM® matrix) adjuvants are preferred choices according to the invention, especially since it has been shown that this type of adjuvants are capable of up-regulating MHC Class II expression by APCs. An ISCOM® matrix consists of (optionally fractionated) saponins (triterpenoids) from Quillaja saponaria, cholesterol, and phospholipid. When admixed with the immunogenic protein, the resulting particulate formulation is what is known as an ISCOM particle where the saponin constitutes 60-70% w/w, the cholesterol and phospholipid 10-15% w/w, and the protein 10-15% w/w. Details relating to composition and use of immunostimulating complexes can e.g. be found in the above-mentioned text-books

dealing with adjuvants, but also Morein B et al., 1995, Clin. Immunother. 3: 461-475 as well as Barr IG and Mitchell GF, 1996, Immunol. and Cell Biol. 74: 8-25 (both incorporated by reference herein) provide useful instructions for the preparation of complete immunostimulating complexes.

Another highly interesting (and thus, preferred) possibility of achieving adjuvant effect is to employ the technique described in Gosselin et al., 1992 (which is hereby incorporated by reference herein). In brief, the presentation of a relevant antigen such as a modified CEA polypeptide of the present invention can be enhanced by conjugating the antigen to antibodies (or antigen binding antibody fragments) against the Fcγ receptors on monocytes/macrophages. Especially conjugates including anti-FcγRI or FcγRI binding structures are believed to enhance immunogenicity for the purposes of vaccination.

Other possibilities involve the use of the targeting and immune modulating substances (i.a. cytokines) mentioned above as candidates for the first and second moieties in the chimeric binding protein. In this connection, also synthetic inducers of cytokines like poly I:C are possibilities.

Suitable mycobacterial derivatives are selected from the group consisting of muramyl dipeptide, complete Freund's adjuvant, RIBI, and a diester of trehalose such as TDM and TDE.

Suitable immune targeting adjuvants are selected from the group consisting of CD40 ligand and CD40 antibodies or specifically binding fragments thereof (cf. the discussion above), mannose, a Fab fragment, and CTLA-4.

Suitable polymer adjuvants are selected from the group consisting of a carbohydrate such as dextran, PEG, starch, man-

nan, and mannose; a plastic polymer; and latex such as latex beads.

Yet another interesting way of modulating an immune response is to include the immunogen (optionally together with adjuvants and pharmaceutically acceptable carriers and vehicles) in a "virtual lymph node" (VLN) (a proprietary medical device developed by ImmunoTherapy, Inc., 360 Lexington Avenue, New York, NY 10017-6501). The VLN (a thin tubular device) mimics the structure and function of a lymph node. Insertion of a VLN under the skin creates a site of sterile inflammation with an upsurge of cytokines and chemokines. T- and B-cells as well as APCs rapidly respond to the danger signals, home to the inflamed site and accumulate inside the porous matrix of the VLN. It has been shown that the necessary antigen dose required to mount an immune response to an antigen is reduced when using the VLN and that immune protection conferred by vaccination using a VLN surpassed conventional immunization using Ribi as an adjuvant. The technology is *i.a.* described briefly in Gelber C *et al.*, 1998, "Elicitation of Robust Cellular and Humeral Immune Responses to Small Amounts of Immunogens Using a Novel Medical Device Designated the Virtual Lymph Node", in: "From the Laboratory to the Clinic, Book of Abstracts, October 12th - 15th 1998, Seascape Resort, Aptos, California".

It is expected that the vaccine should be administered at least once a year, such as at least 1, 2, 3, 4, 5, 6, and 12 times a year. More specifically, 1-12 times per year is expected, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 times a year to an individual in need thereof. It has previously been shown that the memory immunity induced by the use of the preferred autovaccines according to the invention is not per-

manent, and therefore the immune system needs to be periodically challenged with the chimeric binding protein.

Due to genetic variation, different individuals may react with immune responses of varying strength to the same polypeptide. Therefore, the vaccine according to the invention may comprise several different chimeric binding proteins polypeptides in order to increase the immune response, cf. also the discussion above concerning the choice of foreign T_H epitope introductions. The vaccine may comprise two or more polypeptides, where all of the polypeptides are as defined above.

The vaccine may consequently comprise 3-20 different modified polypeptides, such as 3-10 different polypeptides. However, normally the number of peptides will be sought kept to a minimum such as 1 or 2 peptides.

Live vaccines

The second alternative for effecting presentation to the immune system is the use of live vaccine technology. In live vaccination, presentation to the immune system is effected by administering, to the animal, a non-pathogenic microorganism which has been transformed with a nucleic acid fragment encoding the chimeric binding protein. Alternatively, the microorganism is transformed with a vector incorporating such a nucleic acid fragment. The non-pathogenic microorganism can be any suitable attenuated bacterial strain (attenuated by means of passaging or by means of removal of pathogenic expression products by recombinant DNA technology), e.g. *Mycobacterium bovis* BCG., non-pathogenic *Streptococcus* spp., *E. coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella*, etc. Reviews dealing with preparation of state-of-the-art live

vaccines can e.g. be found in Saliou P, 1995, Rev. Prat. 45: 1492-1496 and Walker PD, 1992, Vaccine 10: 977-990, both incorporated by reference herein. For details about the nucleic acid fragments and vectors used in such live vaccines, cf. the discussion below.

Especially BCG has been used extensively as a live bacterial vaccine: The BCG vaccine has been successfully used to prevent tuberculosis around the world. Vaccination can be given after birth and results in few severe complications, even in individuals who are infected with human immunodeficiency virus type 1. BCG possesses strong immune adjuvant activity, and has been used extensively in the treatment of superficial bladder cancers.

As for the polypeptide vaccine, the T_H epitope and/or the first and/or second and/or third moieties can, if present, be in the form of fusion partners to the amino acid sequence derived from the scaffold protein structure.

As an alternative to bacterial live vaccines, the nucleic acid fragment of the invention discussed below can be incorporated in a non-virulent viral vaccine vector. Feasible viral vectors are selected from a pox virus such as vaccinia, MVA (modified Vaccinia virus), canary pox, avi-pox, and chicken pox etc. Alternatively, a herpes simplex virus variant can be used.

Normally, the non-pathogenic microorganism or virus is administered only once to the animal, but in certain cases it may be necessary to administer the microorganism more than once in a lifetime.

Also, the microorganism can be transformed with nucleic acid(s) containing regions encoding the 1st, 2nd and/or 3rd

moieties, e.g. in the form of the immunomodulating substances described above such as the cytokines discussed as useful adjuvants. A preferred version of this embodiment encompasses having the coding region for the chimeric binding protein and the coding region for the immunomodulator in different open reading frames or at least under the control of different promoters. Thereby it is avoided that the chimeric binding protein are produced as fusion partners to the immunomodulator, and effect that may sometimes provide for advantages. Alternatively, two distinct nucleotide fragments can be used as transforming agents.

Nucleic acid vaccination

As an alternative to classic administration of a peptide-based vaccine, the technology of nucleic acid vaccination (also known as "nucleic acid immunisation", "genetic immunisation", "gene immunisation" and "DNA vaccination) offers a number of attractive features.

First, in contrast to the traditional vaccine approach, nucleic acid vaccination does not require resource consuming large-scale production of the immunogenic agent (e.g. in the form of industrial scale fermentation of microorganisms producing the chimeric binding proteins necessary in polypeptide vaccination). Furthermore, there is no need to device purification and refolding schemes for the immunogen. And finally, since nucleic acid vaccination relies on the biochemical apparatus of the vaccinated individual in order to produce the expression product of the nucleic acid introduced, the optimum posttranslational processing of the expression product is expected to occur; this is especially important in the case of autovaccination, since, as mentioned above, a significant fraction of the original B-cell epitopes should be

preserved in the chimeric binding proteins derived from extracellularly exposed polypeptide sequences, and since B-cell epitopes in principle can be constituted by parts of any (bio)molecule (e.g. carbohydrate, lipid, protein etc.). Therefore, native glycosylation and lipidation patterns of the immunogen may very well be of importance for the overall immunogenicity and this is best ensured by having the host producing the immunogen.

Hence, an important embodiment of the method of the invention involves that presentation is effected by administering a nucleic acid fragment encoding and expressing a chimeric binding protein of the invention.

As for the traditional vaccination approach, the nucleic acid vaccination can be combined with *in vivo* introduction, into the APC, of at least one nucleic acid fragment encoding and expressing the second analogue. The considerations pertaining to 1st, 2nd and 3rd moieties and T_H epitopes apply also here.

In this embodiment, the introduced nucleic acid is preferably DNA which can be in the form of naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, emulsified DNA, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, and DNA formulated with an adjuvant. In this context it is noted that practically all considerations pertaining to the use of adjuvants in traditional vaccine formulation apply for the formulation of DNA vaccines. Hence, all disclosures herein which relate to use of adjuvants in the context of polypeptide based vaccines apply *mutatis mutandis* to their use in nucleic acid

vaccination technology. The same holds true for other considerations relating to formulation and mode and route of administration and, hence, also these considerations discussed above in connection with a traditional vaccine apply *mutatis mutandis* to their use in nucleic acid vaccination technology.

One especially preferred type of formulation of nucleic acid vaccines are microparticles containing the DNA. Suitable microparticles are e.g. described in WO 98/31398.

Furthermore, the nucleic acid(s) used as an immunization agent can contain regions encoding the 1st, 2nd and/or 3rd moieties, e.g. in the form of the immunomodulating substances described above such as the cytokines discussed as useful adjuvants. A preferred version of this embodiment encompasses having the coding region for the chimeric binding protein and the coding region for the immunomodulator in different open reading frames or at least under the control of different promoters. Thereby it is avoided that the chimeric binding protein is produced as a fusion partner to the immunomodulator, an effect that is believed to provide advantages in terms of preservation of structure of the scaffold protein structure. Alternatively, two distinct nucleotide fragments can be used, but this is less preferred because of the advantage of ensured co-expression when having both coding regions included in the same molecule.

Under normal circumstances, the nucleic acid of the vaccine is introduced in the form of a vector wherein expression is under control of a viral promoter. For more detailed discussions of vectors according to the invention, cf. the discussion below. Also, detailed disclosures relating to the formulation and use of nucleic acid vaccines are available, cf. Donnelly JJ et al, 1997, Annu. Rev. Immunol. 15: 617-648 and Donnelly JJ et al.,

1997, Life Sciences 60: 163-172. Both of these references are incorporated by reference herein.

The expression cassette in the nucleic acid vaccine can be constructed so as to ensure that no export of the expression product takes place (e.g. by omitting signal sequences that would result in membrane integration or secretion). In this way, only minute amounts of expression product will be exported, whereas the remainder will be processed and presented as peptide fragments in the context of MHC molecules.

Combination of approaches

Various prime-boost strategies have proven effective in facilitating an improved immune response. Hence, according to the present invention, use of any combination of nucleic acid vaccination, live vaccination and polypeptide vaccination may be utilised. However, it is especially preferred to prime via nucleic acid or viral vaccination and boost with a polypeptide vaccine, preferably where the polypeptide vaccine contains the expression product from the nucleic acid of the priming vaccine.

Methods for identifying and preparing the chimeric binding proteins

As has been discussed above, the most important features of the chimeric binding proteins of the invention are their ability to elicit an immune response that is only *effective* in targeting the B-cell epitope of interest even though they might be able to induce antibodies reactive with both the scaffold protein structure and the tolerance breaking amino acid sequence.

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It will not be altogether simple to engineer the chimeric binding protein if an assay for effective presentation of the B-cell epitope is not introduced. Since B-cell epitopes may have very varying structures, the scaffold protein structure that is suitable for one B-cell epitope and its presentation is almost with certainty not suitable for a different B-cell epitope, so the present invention is best put into practice when at least an effective combination of B-cell epitope and scaffold protein structure has been identified.

Hence, the method for identifying the chimeric binding protein of the invention will in its broadest scope include the following steps:

1) providing a first molecule, which binds to a self-antigen of interest in an animal and which includes the first receptor,

2) screening a library of second molecules for their ability to selectively bind to said first receptor of said first molecule,

3) isolating the members of the library that selectively binds in step 2, and

4) preparing, by means of synthesis or recombinant technology, the chimeric binding protein that contains at least a) the binding site of a member isolated in step 3, b) a scaffold protein structure autologous in the animal that stabilises the native 3D structure of said binding site, and c) a non-human MHC Class II binding amino acid sequence; or

i) preparing, by means of synthesis or recombinant technology, a chimeric binding protein containing 1) the second receptor or a mimotope thereof in correct, native 3D conformation, 2) a

scaffold protein structure autologous in the animal, said scaffold protein structure stabilising said 3D conformation and being derived from another molecule in the animal than the second receptor, and 3) the tolerance breaking amino acid sequence.

There are several ways of preparing such chimeric binding proteins of the invention. As discussed for the B-cell epitope part, that part of the chimeric binding molecule can simply be the (already identified) epitope of interest in the relevant self-antigen that is targeted by the vaccines of the present invention - this is normally easiest to achieve when the epitope is a linear peptidic epitope. Under such circumstances, the B-cell epitope can be inserted in a suitable abundant autologous protein structure, or it can be introduced randomly in a putative scaffold structure and subsequently the thus obtained library can be screened for those members that selectively bind to the first receptor. Thus, in this embodiment, the above mentioned library consists of a known putative scaffold protein structure (where the tolerance breaking amino acid sequence may already be present) where the B-cell epitope or a mimotope thereof is shuffled around in various random positions - from this library, only those members that provide a suitable binding to the first receptor (for instance a suitable mAB) will be selected.

Alternatively, the position for introduction of the B-cell epitope can be fixed, whereas the B-cell epitope itself is identified from a library of random sequences inserted in this position. This can preferably be achieved by screening a library of antibodies of the same class and subclass for members that bind to the first receptor. Thus, in this embodiment, the above-mentioned library consists of a known putative scaffold protein structure (where the tolerance

breaking amino acid sequence may already be present) where the B-cell epitope is a member of a random sequence library introduced in the scaffold protein structure at a fixed position. From this library, only those members that provide a suitable binding to the first receptor (for instance a suitable mAB) will be selected.

A third possibility, that does not necessarily require the above-mentioned screening, is to isolate naturally occurring anti-idiotypic antibodies that mimic an epitope of an antigen of interest. For instance, if an animal harbours antibodies against an autologous antigen, then the same animal also harbours anti-idiotypic antibodies reactive with the idiotype of these antibodies. Such anti-idiotypic antibodies can be isolated, sequenced and subsequently a modified version wherein a tolerance breaking amino acid sequence is introduced can be prepared. Again, the resulting molecule will include a B-cell epitope (the idiotype of the anti-idiotypic antibody), a scaffold protein structure (the non-idiotypic region of the anti-idiotypic antibody) and a tolerance breaking amino acid sequence.

One specialised version of this third possibility utilises the following strategy: The first molecule, including the first receptor, is used as an immunogen to immunize a transgenic animal that expresses antibodies autologous in the host harbouring the antigen with the second receptor - if necessary the first molecule may be coupled to an immunogenic carrier molecule such as tetanus toxoid, KLH, and diphtheria toxoid (this is the case if the first molecule is autologous in the transgenic animal). The transgenic animal may for instance be a non-human animal that expresses human immunoglobulin (e.g. as disclosed in WO 93/12227, US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US

5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, WO 91/10741 and WO 01/25492). The first molecule will in this situation induce production of human antibodies that react with the first molecule. By using standard technology for preparing hybridomas (following the teachings of Köhler and Millstein), a selection of hybridomas are prepared that produce monoclonal antibodies reacting with the first molecule, cf. below. These human monoclonal antibodies are thereafter screened for their specific binding to the first receptor so as to exclude those mABs that bind non-specifically to other parts of the first molecule. The hybridomas producing the specifically binding mABs are then isolated and by use of standard cloning and molecular biology technology, the DNA encoding these antibodies (or fragments thereof) is used to transform suitable production cell lines. With respect to the tolerance breaking amino acid sequence, this can either be introduced as part of the cloning steps when the DNA is transformed into the production cell line, but an alternative is to provide a transgenic animal where the IgG molecules already include an amino acid sequence that will be tolerance breaking in the animal to ultimately be vaccinated with the mABs.

In fact, in all the above-referenced embodiments of the inventive method, it is possible to introduce the tolerance breaking amino acid sequence at a later stage, *i.e.* after the combination of B-cell epitope and scaffold protein structure has been identified. However, it is also possible to include the tolerance breaking amino acid sequence so that it is present in the screening steps, when applicable.

In the above-referenced method for identifying a suitable chimeric binding protein of the invention, the first molecule is preferably an antibody and even more preferably a

monoclonal antibody. As already detailed above, this allows for preparation of e.g. an immunogenized anti-idiotypic antibody that will only raise a relevant immune response against the idiotype. Hence, it is most preferred that the first receptor is the idiotype of the antibody.

It will be understood that steps 2 and 3 of the method provides for isolation of those members of the library that interacts specifically with the first receptor. In the event that the receptor is part of a larger molecule (e.g. when the receptor is the antigen binding part of an antibody) then the screening will also give rise to a number of isolated/identified members that react with the remainder of the larger molecule. To exclude these irrelevantly interacting members, it is according to the invention preferred that screening in step 3 includes an exclusion step that allows identification of members of the library that bind the first molecule outside the first receptor so as to exclude such members from subsequent steps. Such an exclusion step involves

a) a test of the library members' ability to bind to the region outside the first receptor in the first molecule, so as to allow exclusion of library members that exhibit such binding, or

b) a test of the library members' ability to compete with the second receptor for binding to the first receptor that allows exclusion of library members that do not exhibit such ability.

In other words, option (a) relies on capture of members of the library that bind to the scaffold protein structure and/or the tolerance breaking amino acid sequence, e.g. in a chromatographic step where the first molecule lacking the first receptor (such as use of an irrelevant antibody of the same subclass as the antibody used as the first molecule) is fixed to a chromatographic medium in a column and where the

library is passed through said column: only members that are not retained by the column will be those that bind the first receptor.

Option (b) relies on competition assays, where only those members of the library are selected that are capable of competing with the relevant second receptor for binding to the first receptor. This can also be done chromatographically, where the first molecule (including the first receptor) is fixed to a chromatographic medium in a column, a molecule including the second receptor is applied to the column in excess so as to block all first receptors, and finally the library members are passed through the column. Only those that flow through will be those that bind the first receptor.

One suitable method for presenting the library of second molecules is to utilise phage display, cf. the example below, but also the technologies of ribosome display, mRNA-display, and yeast surface display are possibilities.

EXAMPLE

The Phage Display Technology

The present invention is illustrated by means of phage display technology as an example - it will be understood, however that any art-recognized method of screening a library of binding partners may be utilised according to the present invention.

The phage display technology is used to provide a direct physical link between phenotype and genotype by utilising the ability to display peptides and proteins on the surface of the filamentous phage.

The technology takes advantage of the infectious ability of the filamentous bacteriophage (like f1 and M13), which infects bacteria by interaction with the F-pilus on the surface of the bacteria. Unlike most bacterial viruses the filamentous phage are not released by cell lysis but is secreted from the cell while the phage is packaged and assembled in the bacterial membrane. The growth of the bacterial cell is only slightly reduced by the infection and it is therefore possible to achieve very high phage titers in culture. (Markowski and Russel 1997).

The filamentous phage protein sheath is made up of five different coating proteins (pIII, pVI, pVII, pVIII and pIX) as illustrated in Fig. 1. In order to display peptides or proteins on the surface of the phage, the coding sequence of the peptide/protein has to be joined with the coding sequence of a coating protein of the phage. George Smith was the first who cloned small protein-encoding DNA-fragments into gIII (the coding sequence of the minor coat protein, pIII) of the phage genome and showed that the resulting phage carried polypeptides that could be recognised by a specific antibody (Smith GP 1985). Later, several proteins have been demonstrated to be functional when displayed on the phage e.g. *E. coli* alkaline phosphatase (McCafferty et al. 1991), human growth hormone (Bass et al. 1990), β-lactamase and antibody fragments (McCafferty et al. 1990 and Huse et al. 1989).

Several antibody libraries have also been displayed on the filamentous phage - e.g. an antibody library was cloned using the entire phage genome as vector (Clackson et al. 1991). As an alternative, the use of phagemid vectors have been widely used. Phagemids are plasmids, which bear the integenic region of a filamentous phage. This region contains a DNA replication origin and a DNA packaging signal. When phagemids are

propagated in cells superinfected with a helper phage, the phagemid DNA is packaged in phage particles in a fashion identical to the phage genome itself. For display purposes the phagemid carries the coding sequence of a coating protein of the phage (usually pIII or part of pIII) to which the antibody-fragment is fused. Upon superinfection with helper phage, antibody fragments are displayed on the surface of the phage, fused to the coating protein. (Hoogenboom et al.1991, Ørum et al. 1993).

Construction of antibody libraries

Commercial antibody libraries for phage display are available (e.g human phage antibody libraries from Dyax). Methods for creating libraries have also been described (Engberg et al. 1996 and Krebber et al. 1997). Construction of an antibody library usually sets out with isolation of mRNA from a source containing antibody-producing cells. (Winter *et al.* 1994). The mRNA is subsequently used as template for cDNA synthesis by RT-PCR. The PCR amplification of the antibody repertoire is done by use of specific primers designed for amplification of the heavy and light chain genes of the antibody fragment. Subsequently the amplified heavy and light chain genes are cloned into a vector and transformed into an *E. coli* strain that expresses F-pili on the surface. The transformed cells are propagated in liquid culture or on plates, and the propagated cells thereby constitute the antibody library. (Engberg et al. 1996, Krebber et al. 1997).

According to the present invention, the most preferred library to display would be a human antibody library, but also antibody libraries from other species would be useful. In that event, the hypervariable regions of the identified antibodies

will be identified and substituted into the hypervariable region of a human antibody scaffold.

Biopanning

In order to prapare the phage display antibody library, the antibody library needs to be superinfected by a helper phage. Following the superinfection the infected bacteria produce recombinant phage particles where each phage represents a unique antibody encoded by genes in the same phage particle. The display of antibody fragments allows for selection and purification of antigen-specific phage by repeated rounds of selection (biopanning or panning).

As shown in Fig. 2, one round of selection consists of: superinfection of the *E. coli* antibody library, production of recombinant phage particles, incubation of phage with the antigen (for instance a monoclonal antibody of choice), removal of non-binding phage by a washing procedure, Elution of bound phage particles and transduction fresh *E. coli* cells with the eluted phage. Elution of the bound phage is generally achieved with low pH buffers, but it is also possible to elute with high pH buffers, soluble antigen or by proteolytic cleavage. In the latter case, a specific protease recognition site has been inserted in the vector between the sequence of the coating protein and the antibody-fragment. (Johansen et al. 1995). After several panning-rounds individual clones are isolated and analysed for binding affinity and specificity for the selected antigen.

Selection strategies

The antigen (which can be any of the above-discussed monoclonal antibodies that have proven useful in a clinical

setting, *i.a.* Herceptin or Retuximab) can be immobilized onto different solid phases such as ELISA wells, latex- or magnetic beads, immunotubes or columns. For cell surface antigens, phage could be directly selected on cells, or antigen-displaying cells may be sorted by virtue of a marker unique for these cells e.g. using fluorescence activated cell sorting (FACS). (Hoogenboom 1997). Combinations and alterations between these methods may be done in order to avoid background binders (phage binding to the immobilization medium) e.g. in the first panning round the antigen is immobilized on ELISA-wells, and in the second round of panning it is immobilized on latex-beads. This method is called alternating panning (Andersen et al. 1996). Another way to avoid non-specific binding is to use a pre-incubation step with an irrelevant antigen e.g. if an antibody-fragment with specificity of a MHC-peptide complex is wanted, an empty MHC-complex is added to the phage-library prior to the panning, this should remove phage with specificity for other regions than the MHC-peptide complex. (Krogsgaard et al. 2000). Binders can usually be isolated after three to four rounds of panning dependent of the panning procedure.

Furthermore, since the present invention aims at isolating only the phage that bind the interesting region interacting with a known receptor structure in the animal to be vaccinated, it is expedient to also remove phages that bind to other regions in the antigen. For instance, if the antigen is a monoclonal antibody, and it is only of relevance to isolate phages that bind to the idiotype of the monoclonal, then a second round of selection can be carried out, where only phages are selected that compete with the monoclonal antibody's target ligand.

This can be done by pre-incubation of the mAB bound to beads in a chromatography column with the phage particles, followed by a wash step that remove non-binding phage. After that, an excess of ligand to the mAB is added and phage that are displaced due to competition can be eluted.

Alternatively, phage that have already passed the step of binding to the antigen can be applied to a column where the antigen has been pre-incubated with its known ligand. Phage that run through the column must be assumed to bind the site occupied by the known ligand.

After identification and isolation of phage that bind the antigen (=the first receptor), the chimeric binding protein can be readily prepared.

Depending on the nature of the antibody library that has been screened, the steps for providing the chimeric binding protein will involve simple isolation and purification of the positive antibodies (in cases where the library screened includes antibodies or fragments that also include the tolerance breaking amino acid sequence), genetic engineering of the DNA encoding the positive antibodies so as to introduce the tolerance breaking amino acid sequence and/or necessary parts of the scaffold protein structure (in cases where only fragments of antibody has been screened and it is necessary to supply all or some part of the remaining scaffold protein structure), and genetic engineering of the DNA encoding the positive antibodies so as to move the antigen binding region to a suitable scaffold protein structure with a tolerance breaking amino acid sequence.

As also mentioned, even though the present example has put focus on phage display of antibodies, any abundant molecule would be suitable, notably albumin.

Other methods for display of peptides or proteins

There exists a number of other methods for identifying specific binding partners that can be used according to the present invention's principles for providing chimeric binding molecules. For instance, the technologies of ribosome display (Mattheakis, L.C. et al. (1994) Hanes, J. and Plückthun, A. (1997)), mRNA-display (Roberts, R.W. and Szostak, J.W. (1997) Liu, R. et al. (2000)), and yeast surface display (Boder E.T and Wittrup KD (1997)) are all feasible alternatives to the phage display technology described herein. Further, the technologies for isolating TSAR's (totally synthetic affinity reagents) that are disclosed in e.g. US 5,498,538 would also serve the purposes of the present invention as would yeast two-hybrid technologies, notably the one described in 6,074,829 where false positive interactions are screened out.

A specific example: Induction of Xolair competing Abs

The monoclonal antibody Xolair is known to bind IgE without being able to cross-link IgE when bound to the FcεRI receptor on mast cells and basophils. In order to produce a chimeric binding protein of the invention, the following simple steps could be carried out:

- Obtaining Xolair
- Generating/obtaining a fully human IgG phage display library (potentially containing suitable epitopes such as the P2 and P30 epitopes from tetanus toxoid or the above-reference PADRE epitope),

- Probing in the phage display with Xolair for antibodies that: 1) compete with human IgE's binding to Xolair (positive selection) and/or 2) does not recognise non-specific IgG (negative selection)
- Immunising humans with selected anti-idiotypic anti-Xolair antibodies (including P2 & P30 or PADRE)
- Testing specificity and safety of generated Ab response.

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CLAIMS

1. A chimeric binding protein that is immunogenic in an animal, said chimeric binding protein being one that binds specifically to a first receptor, said first receptor being one that binds a second receptor present in an antigen of said animal, wherein said chimeric binding protein comprises:
- a B-cell epitope in the form of a binding site that specifically binds the first receptor and which competes with the second receptor for binding to the first receptor,
- a scaffold protein structure that stabilises the 3D conformation of the binding site, said scaffold protein structure being autologous in said mammal, and
- at least one tolerance breaking amino acid sequence, which is heterologous in said animal and which binds to an MHC Class II molecule in said animal.

2. The chimeric binding protein according to claim 1, wherein said scaffold protein structure is derived from an abundant protein, preferably an abundant serum protein.

3. The chimeric binding protein according to claim 1 or 2 wherein said scaffold protein structure is derived from albumin, an immunoglobulin, transferrin, and α_2-macroglobulin.

4. The chimeric binding protein of any one of claims 1-3, wherein said scaffold protein structure is derived from IgG.

5. The chimeric binding protein of any one of claims 1-4, wherein said scaffold protein structure is derived from the non-idiotypic region of a molecule selected from the group consisting of a complete antibody and a fragment thereof such as an $F(ab')_2$ fragment, an Fab fragment, and an scFv.

6. The chimeric binding protein of any one of claims 1-5, wherein said scaffold protein structure comprises a substantially complete amino acid sequence of a polypeptide autologous in said animal.

7. The chimeric binding protein of any one of claims 1-6, wherein said scaffold protein structure comprises a substantial number of B-cell epitope found in the autologous scaffold protein structure in the animal.

8. The chimeric binding protein of any one of claims 1-7, wherein said scaffold protein structure has substantially the same tertiary structure of a polypeptide autologous in said animal.

9. The chimeric binding protein of any one of claims 1-8, wherein said B-cell epitope is constituted by the idiotype of an antibody.

10. The chimeric binding protein of any one of claims 1-9, wherein said first receptor is the idiotype of an antibody or a specific binding region of a ligand that binds the second receptor in said animal.

11. The chimeric binding protein of any one of claims 1-10, wherein said first receptor is the idiotype of a monoclonal antibody.

12. The chimeric binding protein of any one of claims 1-11, wherein said tolerance breaking amino acid sequence is introduced by means of amino acid insertion or substitution in the amino acid sequence of the scaffold protein structure.

13. The chimeric binding protein of any one of claims 1-12, wherein the animal is a human being.

14. The chimeric binding protein of any one of claims 1-13, which is an anti-idiotypic antibody or an effectively binding fragment thereof that is modified so as to include said tolerance breaking amino acid sequence.

15. The chimeric binding protein of any one of claims 1-14, wherein the antigen of said animal that includes said second receptor is selected from the group consisting of immunoglobulin E, CD20, CD11a, beta amyloid, HER-2, and TNFα.

16. The chimeric binding protein of any one of claims 1-15, which further comprises
- at least one first moiety which effects targeting of the chimeric binding protein to an antigen presenting cell (APC) or a B-lymphocyte, and/or
- at least one second moiety which stimulates the immune system, and/or
- at least one third moiety which optimises presentation of the chimeric binding protein to the immune system.

17. The chimeric binding protein according to claim 16, wherein the tolerance breaking amino acid sequence and/or the first and/or the second and/or the third moiety is/are present in the chimeric binding protein by being bound to suitable side groups in the scaffold protein structure.

18. The chimeric binding protein according to claim 17, wherein the tolerance breaking amino acid sequence and/or the first and/or the second and/or the third moiety is/are present in the scaffold protein structure by at least one amino acid substitution and/or deletion and/or insertion and/or addition.

19. The chimeric binding protein according to any one of claims 1-18, wherein the tolerance breaking amino acid

sequence is promiscuous in the animal species to which said animal belongs.

20. The chimeric binding protein according to any one of claims 1-19, wherein the tolerance breading amino acid sequence is selected from a natural promiscuous T helper cell epitope and an artificial MHC-II binding peptide sequence.

21. The chimeric binding protein according to claim 20, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemagluttinin epitope, and a *P. falciparum* CS epitope, and wherein the articial MHC-II binding peptide sequence is a PADRE peptide.

22. The chimeric binding protein according to any one of claims 16-21, wherein the first moiety is a substantially specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen, such as a hapten or a carbohydrate for which there is a receptor on the B-lymphocyte or the APC.

23. The chimeric binding protein according to any one of claims 16-22, wherein the second moiety is selected from a cytokine and a heat-shock protein.

24. The chimeric binding protein according to claim 23, wherein the cytokine is selected from, or is an effective part of, interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF), and the heat-shock protein is

selected from, or is an effective part of any of, HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT).

25. The chimeric binding protein according to any one of claims 16-24, wherein the third moiety is of lipid nature, such as a palmitoyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group.

26. A nucleic acid fragment that encodes the chimeric binding protein according to any one of claims 1-25, or a nucleic acid fragment complementary thereto.

27. A vector carrying the nucleic acid fragment according to claim 26, such as a vector that is capable of autonomous replication.

28. The vector according to claim 27, which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

29. The vector according to claim 27 or 28, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 26, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 26, and optionally a terminator.

30. The vector according to any one of claims 27-29 which, when introduced into a host cell, is capable or incapable of being integrated in the host cell genome.

31. The vector according to any one of claims 27-30, wherein a promoter drives expression in a eukaryotic cell and/or in a prokaryotic cell.

32. A transformed cell carrying the vector of any one of claims 27-30, such as a transformed cell which is capable of replicating the nucleic acid fragment according to claim 26.

33. The transformed cell according to claim 32, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell such as an S_2 or an SF cell, a plant cell, and a mammalian cell.

34. The transformed cell according to claim 32-33, which expresses the nucleic acid fragment defined in claim 26, such as a transformed cell, which secretes or carries on its surface, the chimeric binding protein defined in any one of claims 1-25.

35. A composition for inducing production of antibodies against an antigen in the autologous host, the composition comprising
- a chimeric binding protein according to any one of claims 1-25, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant

36. A composition for inducing production of antibodies against an antigen in the autologous host, the composition comprising
- a nucleic acid fragment according to claim 26 or a vector according to any one of claims 27-31, and

- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

37. A stable cell line which carries the vector according to any one of claims 27-31 and which expresses the nucleic acid fragment according to claim 26, and which optionally secretes or carries the chimeric binding protein according to any one of claims 1-25 on its surface.

38. A method for the preparation of the cell according to any one of claims 32-34, the method comprising transforming a host cell with the nucleic acid fragment according to claim 26 or with the vector according to any one of claims 27-31.

39. A method for preparing the chimeric binding protein of any one of claims 1-25, the method comprising the following steps:

1) providing a first molecule, which binds to a self-antigen of interest in an animal and which includes the first receptor,

2) immunizing, with the first molecule optionally coupled to an immunogenic carrier, a transgenic animal that produces antibodies that are autologous in the animal harbouring the self-antigen or that are autologous in the animal harbouring the self-antigen except for the fact that they also include at least one amino acid sequence that breaks tolerance in the animal,

3) preparing and isolating hybridomas that produce antibodies that bind the first molecule,

4) screening the hybridomas of step 3 for their ability to produce antibodies that selectively bind to said first receptor, and

5) transforming a suitable host cell with at least genetic material that encodes antibodies or functional fragments

thereof where the genetic material is isolated from the hybridomas of step 4 that produce selectively binding antibodies,

6) culturing the host cells transformed in step 5 under conditions that facilitate production of at least the antibodies or functional fragments thereof, and recovering the antibodies or functional fragments thereof from the host cell culture.

40. A method for preparing the chimeric binding protein of any one of claims 1-25, the method comprising the following steps:

1) providing a first molecule, which binds to a self-antigen of interest in an animal and which includes the first receptor,

2) screening a library of second molecules for their ability to selectively bind to said first receptor of said first molecule,

3) isolating the members of the library that selectively binds in step 2, and

4) preparing, by means of synthesis or recombinant technology, the chimeric binding protein that contains at least a) the binding site of a member isolated in step 3, b) a scaffold protein structure autologous in the animal that stabilises the native 3D structure of said binding site, and c) a non-human MHC Class II binding amino acid sequence; or

i) preparing, by means of synthesis or recombinant technology, a chimeric binding protein containing 1) the second receptor or a mimotope thereof in correct, native 3D conformation, 2) a scaffold protein structure autologous in the animal, said scaffold protein structure stabilising said 3D conformation and being derived from another molecule in the animal than the second receptor, and 3) the tolerance breaking amino acid sequence.

41. The method according to claim 39 or 40, wherein the first molecule is an antibody, preferably a monoclonal antibody.

42. The method according to claim 41, wherein the first receptor is the idiotype of the antibody.

43. The method according to any one of claims 39-42, wherein the screening in step 3 includes an exclusion step that allows identification of members of the library that bind the first molecule outside the first receptor so as to exclude such members from subsequent steps.

44. The method according to claim 43, wherein said exclusion step involves

a) a test of the library members' ability to bind to the parts of the first molecule that are outside the first receptor, so as to allow exclusion of library members that exhibit such binding, and/or

b) a test of the library members' ability to compete with the second receptor for binding to the first receptor that allows exclusion of library members that do not exhibit such ability.

45. The method according to any one of claims 40-44, insofar as these are dependent on claim 40, wherein step 3 involves phage display of the second molecules.

46. The method according to any one of claims 40-44, insofar as these are dependent on claim 40, wherein step 3 involves that the library of second molecules is subjected to ribosome display, mRNA-display, or yeast surface display.

47. A method for down-regulating a self-antigen or a cell that displays epitopes of said self-antigen in an animal, the method comprising presenting the animal's immune system with an immunogenically effective amount of a chimeric binding

protein according to any one of claims 1-25 so as to induce a specific immune response against the self-antigen that includes in its structure the second receptor defined in claim 1 or 25.

48. The method according to claim 47, wherein an effective amount of the chimeric binding protein is administered to the animal via a route selected from the parenteral route such as the intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

49. The method according to claim 48, wherein the effective amount is between 0.5 µg and 2,000 µg of the chimeric binding protein.

50. The method according to any one of claim 47-49, wherein the chimeric binding protein is contained in a virtual lymph node (VLN) device.

51. The method according to any one of claims 47-50, wherein the chimeric binding protein has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens.

52. The method according to claim 47, wherein presentation of the chimeric binding protein to the immune system is effected by introducing nucleic acid(s) encoding the chimeric binding protein into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

53. The method according to claim 52, wherein the nucleic acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in

liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant.

54. The method according to claim 53, wherein the nucleic acid(s) is/are contained in a VLN device.

55. The method according to any one of claims 47-54, which includes at least one administration/introduction per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations/introductions.

56. The method according to claim 47, wherein presentation to the immune system is effected by administering a non-pathogenic microorganism or virus which is carrying and expressing a nucleic acid fragment according to claim 26.

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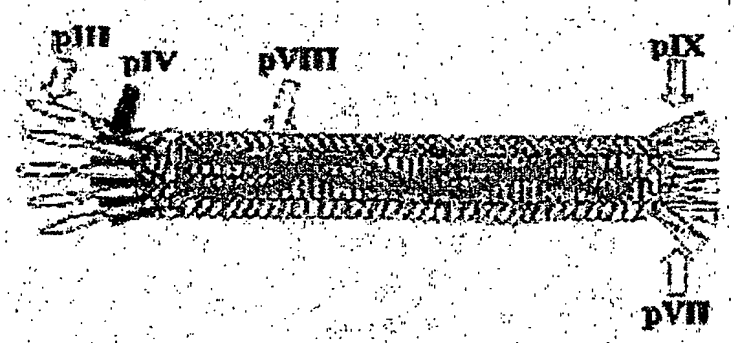

Fig. 1

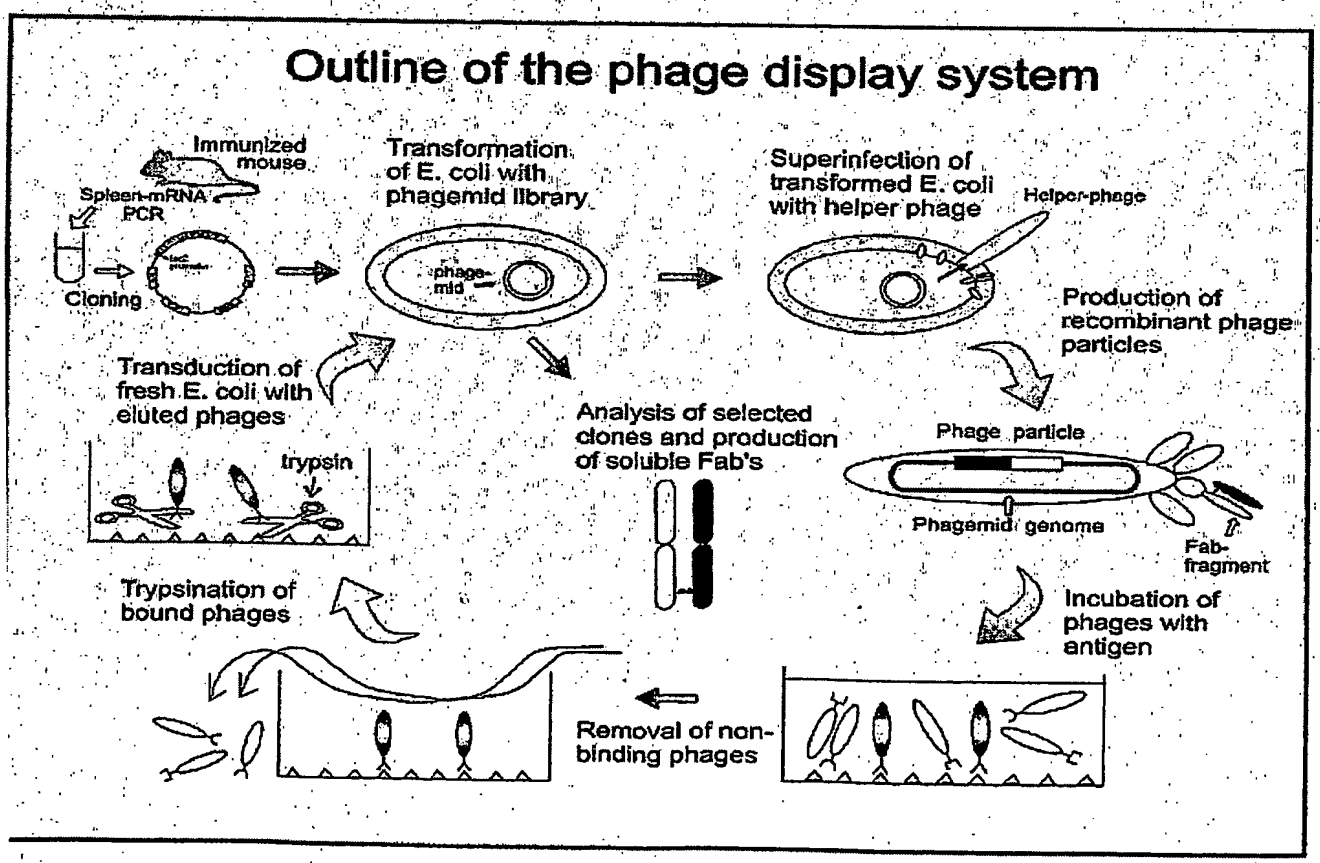

Fig. 2

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